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(54) Title: PERFLUORO-CHEMICAL EMULSION WITH STABILIZED VESICLES (57) Abstract A stable perfluorochemical emulsion is provided which comprises perfluorochemical particles in stabilized vesicles. The vesicles comprise a biocompatible polymer formed by coating the perfluorochemical particles with one or more phospholipid monomer(s) and polymerizing the monomer(s).		

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10 PERFLUOROchemical EMULSION WITH STABILIZED VESICLES

Related Applications

 This application is a continuation-in-part of U.S.
Application Serial No. 07/166,690 filed March 11, 1988,
15 which is incorporated herein by this reference.

Field of the Invention

 The present invention relates to stable perfluoro-
chemical emulsions and to the emulsification techniques
20 and ingredients used in their preparation.

Background of the Invention

 Because perfluorochemicals have the ability to releas-
ably bind oxygen, perfluorochemical preparations have been
25 evaluated for use as blood substitutes and as ischemic
modifiers. Such perfluorochemical preparations are typically
prepared by emulsifying the perfluorochemical compound in
an aqueous medium to form a perfluorochemical emulsion.
Perfluorochemical emulsions are free of infectious agents
30 and antigens, and their use obviates the need for blood
typing of the recipient.

 Although perfluorochemicals are chemically inert,
they appear to adversely affect blood platelets and clotting
factors. This adverse effect is believed to be due to the
35 low surface tension of perfluorochemicals.

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1 In an effort to avoid the adverse effect of perfluoro-
chemicals on blood platelets and clotting factors, per-
fluorochemical emulsion particles are coated with a lipid,
such as lecithin. Emulsions containing lipid-coated
5 perfluorochemical particles are, for example, disclosed
in U.S. Patents Nos. 3,962,439, 4,252,827, 4,423,077 and
4,497,829.

 The prior-art perfluorochemical emulsions do not have
a sufficiently high level of stability to withstand steril-
10 ization at elevated temperatures followed by storage in
the liquid state at room temperature. Thus, their storage
life in an unfrozen state is shorter than desired.

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1 Summary of the Invention

A perfluorochemical emulsion of enhanced stability is therefore provided in accordance with this invention to overcome the problems associated with high temperature
5 sterilization and short shelf life. The emulsion comprises perfluorochemical particles contained within stabilized vesicles which are formed of a biocompatible polymer.

In a preferred embodiment, the method for producing the perfluorochemical emulsions of this invention includes the
10 step of combining a perfluorochemical and a monomeric emulsifying material in the absence of a polymerization initiator to form a mixture. Preferably, the monomeric emulsifying material is a phospholipid monomer wherein each of the acyl chains of the phospholipid comprises a
15 fatty acid moiety selected independently from the group consisting of conjugated di-ene fatty acids, conjugated di-yne fatty acids, conjugated ene-yne fatty acids, and fatty acids containing sulphydryl groups.

The perfluorochemical material and monomeric emulsifying
20 agent are homogenized in an aqueous medium until perfluorochemical particles of a desired size are coated with the monomeric emulsifying material forming an emulsion. Preferably, the diameter of the perfluorochemical particles (including their emulsifier coatings) is less than about
25 0.3 micron. The emulsion is exposed to an appropriate polymerization initiator which causes polymerization of the emulsifying material coating on the perfluorochemical particles. Such polymerization results in the formation of stabilized polymer vesicles which encapsulate or contain
30 the perfluorochemical particles.

In a preferred embodiment, the phospholipid monomer is selected from the group consisting of phosphatidyl-L-cholines, phosphatidyl-L-serines, phosphatidyl-L-ethanolamines, and mixtures thereof. The preferred perfluoro-
35 chemical is selected from the group consisting of perfluoro-

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1 decalin, perfluorotertiary-amines, isoquinolidine per-
fluorochemical derivatives, and mixtures thereof.

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1 Detailed Description of the Invention

A stable aqueous perfluorochemical emulsion is provided in accordance with practice of principles of this invention. The particles of the emulsion comprise one or more perfluorochemical compounds contained within stabilized vesicles which are formed of a biocompatible polymer.

The stable emulsions provided in accordance with this invention can be used as a medium for carrying oxygen to the tissues of a human; for example, they can be administered as a blood substitute or as an ischemic modifier. Because the perfluorocarbon particles are contained within polymerized (stabilized) vesicles, the emulsion is more stable than emulsions which incorporate perfluorochemical particles coated with a non-polymerized emulsifier coating. For example, the emulsions of this invention (1) can withstand higher and longer sterilization temperatures and times; (2) possess greater stability after sterilization which permits longer storage times; and (3) have longer circulating in vivo half-lives compared to emulsions of the same perfluorochemicals which incorporate non-polymerized emulsifier coatings.

In a preferred embodiment, the method for producing the perfluorochemical emulsions of this invention includes the steps of (1) combining a perfluorochemical and a monomeric emulsifying material in the absence of polymerization initiators to form a mixture; (2) homogenizing the mixture in an aqueous medium until perfluorochemical particles of a desired size are coated with the monomeric emulsifying material thereby forming a first emulsion; and (3) exposing the first emulsion to an appropriate polymerization initiator, for example, to ultraviolet radiation. Exposure to the polymerization initiator causes polymerization of the emulsifying material coating on the perfluorochemical particles to provide polymer vesicles which encapsulate or contain the perfluorochemical particles.

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1 The polymer vesicles are substantially more stable than
non-polymerized lipid coatings on the perfluorochemical
particles of standard prior-art emulsions.

The monomeric emulsifying materials useful in practice
5 of this invention are those (1) which are effective to
emulsify the particular perfluorochemical (or mixture of
perfluorochemicals) being used resulting in perfluorochemical
particles having a diameter of less than about 0.3 micron
coated with the emulsifying material and (2) which, after
10 polymerization, provide stabilized perfluorocarbon polymer
vesicles which are biocompatible when administered to a
human in a physiologically acceptable emulsion medium.
The term "biocompatible" as used herein means a lack of
toxic interactions when administered to an animal or human.

15 Although any material which provides for the appropriate
emulsification of the perfluorochemical material being
used and which can be polymerized to form a biocompatible
vesicle, is useful in practice of the present invention, it
is preferred that the monomeric material be a monomeric
20 lipid; preferably, a monomeric phospholipid. Most prefer-
ably, the monomeric lipid is photopolymerizable, i.e., it
is polymerized by means of ultraviolet radiation. The
phospholipids useful in practice of the present invention
incorporate acyl chains which comprise a fatty acid moiety
25 selected independently from the group consisting of con-
jugated di-ene fatty acids, conjugated di-yne fatty acids,
conjugated ene-yne fatty acids, and fatty acids containing
sulphydryl groups. Preferably, both fatty acid moieties
of each phospholipid incorporate the same degree of unsat-
30 uration, i.e., they both contain conjugated di-ene bonds,
or they both contain conjugated di-yne bonds, or they both
contain ene-yne bonds. Preferably, the conjugated bonds
are on the same carbons in both chains.

It is most preferred that the monomeric phospholipid
35 be selected from the group consisting of phosphatidyl-L-

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1 cholines, phosphatidyl-L-serines, phosphatidyl-L-ethanolamines, and mixtures thereof. Preferably, the fatty acid moieties useful in the present invention are those which incorporate from about 10 to about 30 carbon atoms. Such
5 useful acids are, for example, 2,4-octadecadienoic acid, 10,12-tricosadiynoic acid, 10,12-pentacosadiynoic acid, 12-methacryloyloxy dodecanoic acid, 1,2(lipoyl)dodecanoic acid, pentacosatrans-10-ene,12-ynoic acid, and pentacosatrans-12-ene,10-ynoic acids.

10 Monomeric phospholipids useful in accordance with this invention may also be natural phospholipids which have been altered to render them polymerizable. Such alteration, for example, could involve chemical modification to remove some of the natural side chains, followed by
15 chemical modification to place a desired side chain on the molecule followed by purification. The desired side chain would typically comprise a fatty acid having conjugated di-ene bonds, conjugated di-yne bonds, conjugated ene-yne bonds, or containing sulphydryl groups.

20 Non-limiting examples of monomeric phospholipids useful in practice of this invention incorporating di-ene fatty acid moieties include any of the 1,2-di-(X,X+2-dienoyl)-sn-glycero-3-phosphoryl-cholines, such as (1) 1,2-di-(2,4-octadecadienoyl)-sn-glycero-3-phosphoryl-
25 choline. Also useful are (2) Bis[12-(methacryloyloxy)dodecanoyl]-L- α -phosphatidylcholine, (3) 1-[12-methacryloyloxy)dodecanoyl]-2-palmitoyl-L- α -phosphatidylcholine, and (4) 1-palmitoyl-2-[12-(methacryloyloxy)dodecanoyl]-L- α -phosphatidylcholine. The 1,2-di-(2,4-octadecadienoyl)-sn-
30 glycero-3-phosphoryl-choline material can be purchased from Nippon Oil and Fats Company Ltd., of Chiyoda-ku, Tokyo, Japan. Methods for synthesizing the phosphatidyl choline compounds Nos. (2), (3), and (4) are outlined in an article by S. L. Regen et al titled "Polymerized Phosphatidylcholine
35 Vesicles. Synthesis and Characterization," Journal of the

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1 American Chemical Society, 1982, Vol. 104, pp. 791-795,
which is incorporated herein by this reference. Other
dieneoic acids useful in practice of the present invention
are (5) 1,2-di(10,12-hexadecadieneoyl)-sn-glycero-3-phos-
5 phoryl choline. Materials for synthesizing the phosphatidyl
choline compound No. (5) are outlined in B. Hupfer et al,
Makromol. Chem, 1981, 182, p. 247, which is incorporated
herein by this reference.

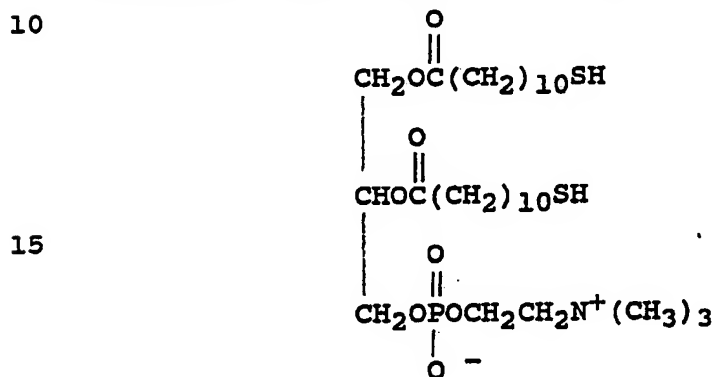
Non-limiting examples of monomeric phospholipids
10 useful in practice of this invention comprising di-yne
fatty acid moieties include any of the 1,2-di-(X,X+2-
diynoyl)-sn-glycero-3-phosphoryl-choline acids or salts,
such as (6) 1,2-di-(10,12-tricosadiynoyl)-sn-glycero-3-
phosphoryl-choline and (7) 1,2-di-(10,12-pentacosadiynoyl)-
15 sn-glycero-3-phosphoryl-choline. Methods for synthesizing
the phosphatidyl choline compounds Nos. (6) and (7) are
disclosed in an article by D.S. Johnston et al titled
"Phospholipid Polymers -- Synthesis and Spectral Charac-
teristics," Biochimica and Biophysica Acta, 602 (1980) pp.
20 57-69, which is incorporated herein by this reference.

Non-limiting examples of monomeric phospholipids
useful in practice of this invention comprising ene-yne
fatty acid moieties include (8) 1,2-di-(tricos-trans-10-
ene-12-ynoyl or tricos-trans-12-ene-10-ynoyl)-sn-glycero-
25 3-phosphoryl-choline or (9) 1,2-di-(pentacos-trans-10-
ene-12-ynoyl or pentacos-trans-12-ene-10-ynoyl)-sn-glycero-
3-phosphoryl-choline. Conjugated ene-yne fatty acids are
made by reduction of one mole of the conjugated di-yne
fatty acids with two moles of sodium in liquid ammonia.
30 This leads to selective reduction of one of the two triple
bonds to a double bond in the trans-configuration. The
resulting product is then purified by vacuum distillation.
The resulting acids will be a mixture of two isomers, for
example, reduction of 10,12-pentacosadiynoic acid by sodium
35 in liquid ammonia would yield pentacos-trans-10-ene, 12-

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1 ynoic acid and pentacosatrans-12-ene, 10-ynoic acid.
 These acids would be used without separation in the preparation of phosphatidyl-L-cholines by the methods disclosed in D.S. Johnston et al, Biochem. Biophys. Acta 602, (1980),
 5 pp. 57-69, which are described above as being useful for the preparation of di-yne derivatives of phosphatidyl-L-choline.

An example of a monomeric phospholipid useful in practice of this invention comprising fatty acid moieties incorporating sulphydryl groups is



20 Methods for synthesizing the above-described sulphydryl-containing monomeric phospholipid, for example, is outlined in an article by S. Regen titled "Polymerized Phosphatidylcholine Vesicles as Drug Carriers," Annals of the New York Academy of Sciences, Vol. 446 (1985), pp. 296-307, which
 25 is incorporated herein by this reference.

The perfluorochemical compounds useful in preparing the emulsions of the present invention include, but are not limited to, those disclosed in U.S. Patents Nos. 3,962,439, 4,252,827, 4,425,347 and 4,596,810, which are
 30 incorporated herein by this reference. Preferred perfluorochemical compounds include perfluorodecalin, perfluorotertiary-amines, such as perfluorotri-n-propylamine, and perfluorotri-n-butylamine, and isoquinolidine derivatives of perfluorochemicals. Perfluorodecalin is particularly
 35 preferred.

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1 The emulsion particles useful in accordance with the
present invention can comprise a single perfluorochemical
or can comprise mixtures of perfluorochemicals. Preferred
perfluorochemical mixtures include (1) perfluorodecalin
5 and perfluorotri-n-propylamine; (2) perfluorotri-n-propyl-
amine and perfluorotri-n-butylamine; (3) perfluoro-N,N'-
dimethylcyclohexylmethyl-amine and perfluorodecalin; (4)
perfluoro-3,3,1-trimethylbicyclo [3.3.1] nonane and per-
fluoro-N,N'-dimethylcyclohexylmethylamine; and (5) perfluoro-
10 chemical isomeric mixtures, such as cis- and trans-perfluoro-
decalin.

Polymerization of the monomeric emulsifying material
may be initiated in a number of ways, depending on the
material. Examples of polymerization initiation stimulation
15 include ultraviolet (UV) radiation, X-ray radiation, heat,
and chemical initiation, for example, using azoisobutyl-
nitrile (AIBN) or azobis-(2-amidinopropane) dihydrochloride
(AAPD). Preferably, the monomeric phospholipids are photo-
polymerizable, i.e., they can be initiated by UV radiation.

20 In one embodiment, the stable emulsion of this inven-
tion is formed by providing a mixture of one or more
monomeric phospholipids and one or more perfluorochemical
materials in water so that the %w/v of the perfluorochemical
material is preferably from about 10% to about 50%, more
25 preferably from about 25% to about 35%, and the %w/v of
the monomeric phospholipid is preferably from about .05%
to about 5%, more preferably from about 1.5% to about
2.5%. The symbol "%w/v" as used herein means the amount of
material by weight in grams based on 100 milliliters of
30 the resulting emulsion. The mixture is held at approximately
room temperature, and nitrogen or carbon dioxide gas is
bubbled through the solution for 15 minutes to saturate
the solution to remove oxygen. The presence of oxygen in
the perfluorochemical/monomeric phospholipid mixture prior
35 to homogenization tends to lead to an undesirably high

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1 level of free fluoride. Accordingly, it is preferred
that oxygen be removed from the mixture prior to homogen-
ization. The oxygen is typically removed by saturating
the mixture, as described above, with nitrogen or carbon
5 dioxide gas.

After the oxygen is removed from the mixture, the
mixture is homogenized with a high shear mixture, such as
a Turbo-mixer under a blanket of inert gas to produce a
crude emulsion. The average particle size of the crude
10 emulsion is measured by inelastic laser light scattering,
using, for example, a Brookhaven BI-90 instrument, provided
by Brookhaven Instruments Co., of Holtsville, New York
11742. Typically, the average particle size of the crude
emulsion is greater than about 1-2 microns. The crude
15 emulsion is then passed through a Manton-Gaulin homogenizer,
or similar high-pressure homogenizer, a sufficient number
of times so that the final particle size is less than a
selected value, for example, less than about 0.3 micron.
Emulsification is done under a nitrogen stream at pressures
20 of 200-600 kg/cm² at temperatures of from about 4°C to
about 80°C, preferably from about 40°C to about 50°C.
During homogenization in the Manton-Gaulin homogenizer,
the particle size of a sample taken at the end of each
pass is measured. When the average particle size reaches
25 a plateau value, usually between 0.05 and 0.16 micron,
typically after 6 or 7 homogenization steps, the emulsifi-
cation is complete.

The perfluorochemical particles (including the coating
of polymerizable monomeric phospholipid) preferably have a
30 diameter less than about 0.3 micron, more preferably are
in the range of from about 0.1 to about 0.3 micron, and most
preferably are from about 0.15 to about 0.2 micron.

Perfluorochemical particles larger than about 0.3
micron in diameter are not desired because such particles
35 tend to be more toxic than smaller particles and are removed

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1 relatively rapidly from the circulation by the reticuloen-
dothelial system (RES). Although emulsion particles smaller
than about 0.1 micron in diameter can be prepared, such
particles are difficult to prepare using current emulsifi-
5 cation and sterilization technology and, hence, are not
preferred. Particles prepared with phospholipid monomers
and the example perfluorochemicals typically have a mean
size of from about 0.1 to about 0.2 micron.

The final size of the emulsion particles is a function
10 of the perfluorochemical or perfluorochemicals used, the
monomeric phospholipid emulsifier, and the energy imparted
to the emulsion during the emulsification process. For
example, emulsions made with perfluorotri-n-butylamine have
smaller particle sizes than emulsions made with perfluoro-
15 decalin, perfluorotri-n-propylamine or combinations thereof.
Although emulsifiers such as Pluronic F-68, a non-ionic
surfactant produced by BASF-Wyandotte, Inc., of Wyandotte,
Michigan, can be used in addition to the above-described
polymerizable (monomeric) phospholipid emulsifiers, it is
20 preferred that the monomeric phospholipid emulsifiers be
used alone. Using only polymerizable (monomeric) emulsifiers
results in a polymerized vesicle which is made up in its
entirety of a polymerized material; whereas, when a non-
polymerizable emulsifier is used in conjunction with the
25 monomeric phospholipid, portions of the vesicle which
encapsulate the perfluorochemical will be unpolymerized.
This results in less stable emulsions.

The stabilized emulsions provided in accordance with
this invention may be isotonic, containing an appropriate
30 amount of sodium chloride or other electrolytes, including
the components in the Ringers solution or lactated Ringers
solution. For that purpose, the presence of glycerine in
an amount of 2.5% (w/v) can be used because glycerine
contributes to the stability, in addition to the isotonicity
35 of the emulsions.

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1 The stabilized perfluorochemical emulsions provided
in accordance with practice of the present invention contain
very finely divided perfluorocarbon particles encapsulated
within stabilized polymeric vesicles. Thus, the particles
5 do not aggregate into coarse particles during storage of the
emulsions for a considerably long time. The emulsions
can be administered to mammals without harm of tissue due
to aggregation.

 The stabilized perfluorochemical emulsions of the
10 present invention can, for example, (1) be administered
intravenously to animals or humans who are in need of
blood; (2) be administered for treating metastasis or
cancerous tumors; (3) be administered to oxygenate tumors
to enhance the effect of radiotherapy; and (4) be admin-
15 istered to oxygenate tissue downstream from the catheter
during angioplasty procedures.

 The present invention is illustrated in greater detail
by the following examples which should not be construed to
20 limit the invention in any way.

Example 1

Preparation of Perfluorochemical Emulsion Using the Polymerizable Monomeric Emulsifier 1,2-di-(10,12-tricosadi- 25 noyl)-sn-glycero-3-phosphoryl-choline.

25 17.5 gm of perfluorodecalin and 7.5 gm of perfluorotri-
n-propylamine (17.5%w/v and 7.5%w/v, respectively) are
mixed together in an aqueous medium with 2.0%w/v 1,2-di-
(10,12-tricosadiynoyl)-sn-glycero-3-phosphoryl-choline.
The mixture is held at approximately room temperature and
30 nitrogen gas is bubbled through the solution for 15 minutes
to saturate the solution and remove oxygen. The mixture
is crudely homogenized with a high shear Turbo-mixer under
a blanket of inert gas (nitrogen) in the absence of UV
light to provide perfluorochemical particles (perfluoro-
35 decalin/perfluorotri-n-propylamine particles) coated with

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1 the 1,2-di-(10,12-tricosadiynoyl)-sn-glycero-3-phosphoryl-
choline emulsifier. The size of the emulsion particles is
measured using a Brookhaven BI-90 instrument and is found
to be greater than about 1 micron in diameter. The crude
5 emulsion is then passed through a Manton-Gaulin homogenizer
under a nitrogen stream at a pressure of 200 to 600 kg/cm²
at about 35°-45°C and collected.

The particle size distribution of the emulsion is
measured after each pass through the homogenizer, and is
10 from about 0.1 to about 0.15 micron in diameter after 7
passes. The resulting emulsion is cooled to below the
fluid-gel transition temperature of the monomeric phospho-
lipid. The transition temperature of 1,2-di-(10,12-tricosa-
diynoyl)-sn-glycero-3-phosphoryl-choline is about 38°C.
15 The 1,2-di-(10,12-tricosadiynoyl)-sn-glycero-3-phosphoryl-
choline coating is then polymerized by exposure of the
emulsion to 254 nm UV light, provided by an R-52 Mineralight,
which has a peak radiation emission of 254 nm and an energy
output of approximately 1200 microwatts/cm² at 15 centimeters
20 from the face for at least 30 minutes. The change in the
degree of polymerization is seen by the color change in
the emulsion or by UV spectral analysis of a sample of the
emulsion. After the monomeric coating is polymerized, the
emulsion is filtered through a 3-micron filter and placed
25 in a glass vial. The vial is then flushed with nitrogen
and sealed. The vial is autoclaved at 121°C for 8 minutes
and is cooled to room temperature. The resulting sterilized
emulsion containing perfluorochemical particles encapsulated
in vesicles of poly-1,2-di-(10,12-tricosadiynoyl)-sn-glycero-
3-phosphoryl-choline is tested for particle size distribu-
30 tion, oxygen capacity, and toxicity.

To test the emulsion for oxygen capacity, a 0.5 ml or
1 ml sample of the non-oxygenated emulsion is taken and
analyzed for carbon dioxide, oxygen, and nitrogen content
35 by the method of Van Slyke. (D.D. Van Slyke and W.C. Stadie,

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1 The Determination of the Gases of the Blood, J. Biol.
Chem., 59(1), 1921, 1-42; D. D. Van Slyke and J.M. Neill,
The Determination of Gases in Blood and Other Solutions by
Vacuum Extraction and Monometric Measurement I, J. Biol.
5 Chem., 61(2), 1921, 523-573. The two articles by Van
Slyke et al are incorporated herein by this reference.)
Oxygen is bubbled through the emulsion for 15 minutes at a
flow rate of 2-3 liters per minute. A 0.5 ml or 1 ml
sample of the resulting oxygenated emulsion is taken, and
10 once again is analyzed for carbon dioxide, oxygen, and
nitrogen content by the method of Van Slyke. The oxygen
gas content after oxygenation less the oxygen gas content
before oxygenation is the net oxygen capacity of the
emulsion. This value is between 4 and 7 vol % oxygen at
15 760 mmHg pressure for a 25%w/v perfluorochemical emulsion.
Alternatively, the samples may be analyzed for oxygen
content in the Lexington Lex-O₂-Con oxygen apparatus
(Lexington Instruments, Waltham, Massachusetts). A 20
microliter sample would be injected into the machine, the
20 oxygen content before and after oxygenation determined,
and the oxygen capacity calculated by difference. These
or other methods will all give comparable results for
oxygen capacity.

The 25% emulsion is diluted to 20%w/v by the addition
25 of one-fifth volume of 4% sodium chloride solution. The
resulting emulsion will be injected intravenously via the
tail veins of Wistar rats at a rate of 1 ml/minute. Groups
of 5-10 animals are given doses of 144, 72, 36, or 18 ml
of emulsion/kg body weight. The animals are observed for
30 acute toxic signs and symptoms, and for body weight gain
and survival after seven days of observation. Animals
are given food and water ad libitum. The emulsion is non-
toxic if the LD₅₀, lethal dose for 50% of the animals, is
greater than about 50 ml/kg.

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1 Example 2

Preparation of Perfluorochemical Emulsion Using the Polymerizable Monomeric Emulsifier 1,2-di-(10,12-pentacosadiynoyl)-sn-glycero-3-phosphoryl-choline.

5 The same procedure that is used for preparing and testing the emulsion of Example 1 is used for Example 2, except that (a) 1,2-di-(10,12-pentacosadiynoyl)-sn-glycero-3-phosphoryl-choline is used in place of 1,2-di-(10,12-tricosadiynoyl)-sn-glycero-3-phosphoryl-choline and (b) the resulting emulsion is cooled to below the fluid-gel
10 transition temperature of 1,2-di-(10,12-pentacosadiynoyl)-sn-glycero-3-phosphoryl-choline, which is about 48°C.

Example 3

15 Preparation of Perfluorochemical Emulsion Using the Polymerizable Monomeric Emulsifier 1,2-di-(2,4-octadecadienoyl)-sn-glycero-3-phosphoryl-choline.

17.5 gm of perfluorodecalin and 7.5 gm of perfluorotri-
n-propylamine (17.5%w/v and 7.5%w/v, respectively) are
mixed together in an aqueous medium with 2.0% w/v 1,2-di-
20 (2,4-octadecadienoyl)-sn-glycero-3-phosphoryl-choline. The mixture is held at approximately room temperature, and carbon dioxide gas is bubbled through the solution for 15 minutes to saturate the solution and to remove oxygen. The mixture is crudely homogenized with a high shear Turbo-
25 mixer under a blanket of carbon dioxide in the absence of polymerization initiators. The size of the emulsion particles is measured using a Brookhaven BI-90 instrument and is found to be greater than about 1-2 microns in diameter. The crude emulsion is then passed through a
30 Manton-Gaulin homogenizer or similar high-pressure homogenizer and collected.

The particle size distribution is measured after each pass through the homogenizer and is about 0.15 micron after 7 passes. The 1,2-di-(2,4-octadecadienoyl)-sn-glycero-
35 3-phosphoryl-choline coating is then polymerized by adding

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1 5 mol % (relative to the phospholipid concentration) of
either Azoisobutylnitrile (AIBN) or azobis-(2-amidino-
propane) dihydrochloride (AAPD) and heating the emulsion
to 60°C for about 30 minutes. The change in the degree of
5 polymerization is seen by UV spectral analysis of a sample
of the emulsion. After the monomeric coating is polymerized,
the emulsion is filtered through a 3-micron filter and
placed in a glass vial. The vial is then flushed with
nitrogen and sealed. The vial is autoclaved at 121°C for
10 8 minutes and is cooled to room temperature. Using the
same procedures used in Example 1, the sterilized, poly-
merized emulsion is tested for particle size distribution
and oxygen capacity. The emulsion is also tested by means
well known in the art for residual AIBN or AAPD, as appro-
15 priate. If the levels are higher than desired, the AIBN
or AAPD is removed by washing, or the like.

Such washing can be accomplished by suspending 100 ml
of the resulting emulsion in 100 ml of 4% sodium chloride
solution and mixed. The mixed solution is then centrifuged
20 at 3,000 x g for approximately 30 minutes. The supernatant
is discarded, and a volume of fresh 4% sodium chloride
equal to the volume of the supernatant (approximately 200
ml) is added. The precipitated emulsion particles are
resuspended in the 4% sodium chloride, and the suspension
25 is once again separated by centrifugation. The washed
emulsion particles are suspended in 100 ml of water for
injection.

Example 4

30 Preparation of Stabilized Emulsion for Intravenous Injection as a Radiation Sensitizer

100 ml of the emulsion prepared in Examples 1, 2, or
3 are diluted by the addition of 25 ml of 4% sodium chloride
solution. The resulting solution is mixed. The emulsion
35 is then administered to tumor-bearing rats at a dose of

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- 1 from about 8-12 ml/kg intravenously via the tail vein.
The rats are then allowed to breathe oxygen for 30 minutes,
prior to and during radiation treatment. The tumor growth
delay or surviving tumor cell fraction is used to determine
5 the radiation sensitization effect.

Example 5

Preparation of Stabilized Emulsion for Use in Percu- taneous Transluminal Coronary Angioplasty

- 10 30 ml of solution 1, comprising 3.5%w/v sodium bicar-
bonate USP, 0.56%w/v potassium chloride USP, in water for
injection USP, and 70 ml of solution 2, comprising 4.29%w/v
sodium chloride USP, 1.29%w/v dextrose USP, anhydrous,
0.305%w/v magnesium chloride - 6H₂O USP, 0.254%w/v calcium
15 chloride - 2H₂O USP, in water for injection USP, are added
to 400 ml of the emulsions prepared in Examples 1, 2, or
3. The solutions are added sequentially and separately,
and the emulsion is mixed after each addition. The compo-
sition of the mixed emulsion ready for administration are
20 14.0 g/100 ml perfluorodecalin, 6.0 g/100 ml perfluorotri-
n-propylamine, .60 g/100 ml sodium chloride USP, 1.6 g/100
ml polymerized phospholipids, .21 g/100 ml sodium bicarbonate
USP, .18 g/100 ml dextrose USP, anhydrous, .043 g/100 ml
magnesium chloride - 6H₂O USP, .036 g/100 ml calcium chloride
25 - 2H₂O USP, .034 mg/100 ml potassium chloride USP, in water
for injection USP.

Example 6

Intravenous Injection of the Stabilized Perfluoro- chemical Emulsion as an Erythrocyte Substitute

- 30 30 ml of annex solution C, comprising 3.5%w/v sodium
bicarbonate USP, 0.56%w/v potassium chloride USP, in water
for injection USP, and 70 ml of annex solution H, comprising
4.29%w/v sodium chloride USP, 3.0 %w/v hydroxylethyl starch,
1.29%w/v dextrose USP, anhydrous, .305%w/v magnesium chloride
35 - 6H₂O USP, .254%w/v calcium chloride - 2H₂O USP, in water

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1 for injection USP, are added to 400 ml of the emulsions
prepared in Examples 1, 2, or 3. The solutions are added
sequentially and separately, and the emulsion is mixed
after each addition. The resulting emulsion is oxygenated
5 by bubbling 1-2 liters per minute of oxygen through the
mixed emulsion for 15-30 minutes. The resulting oxygenated
emulsion is administered to a 150 gram conscious male
Wistar rat. A double lumen catheter is placed in the
right atrium of the rat heart under anesthesia. The rat
10 is allowed to recover to full consciousness. The catheter
is connected to a Harvard Infusion Pump (Harvard Apparatus,
South Natick, Massachusetts) such that one side of the
catheter infuses the oxygenated emulsion into the right
atrium while the other catheter is withdrawing blood from
15 the right atrium. The dose of emulsion is 24 ml. The
animal is placed in a 100% oxygen atmosphere, and the
exchange transfusion is carried out at a flow rate of 1
ml/minute until 24 ml of the emulsion is infused, and 24
ml of the blood-emulsion is removed from the rat. The
20 resulting hematocrit of the rat after the transfusion is
completed is 2-4%. The animal is allowed to breath 100%
oxygen for day 1, 90% oxygen, 10% air for day 2, with the
oxygen decreasing 10% and the air increasing 10% per day
until day 7. The animal is then returned to room air.
25 Animals exchanged with other non-oxygen-carrying materials
to a hematocrit of 2-4% under these conditions will not
survive. This test demonstrates the oxygen-carrying capacity
of the emulsions of Examples 1, 2, and 3 in an in-vivo
erythrocyte replacement test system.

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The above descriptions of preferred embodiments of
stable perfluorochemical emulsions and the emulsification
techniques and ingredients used for their preparation are
for illustrative purposes. Because of variations which
35 will be apparent to those skilled in the art, the present

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1 invention is not intended to be limited to the particular
embodiments described above. The scope of the invention
is defined in the following claims.

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1 WHAT IS CLAIMED IS:

1. A stable perfluorochemical emulsion comprising perfluorochemical particles contained within stabilized
5 vesicles, the stabilized vesicles comprising a biocompatible polymer formed by coating the perfluorochemical particles with one or more phospholipid monomers and polymerizing the monomers.

10 2. A stable perfluorochemical emulsion as claimed in claim 1 wherein each of the acyl chains of such a phospholipid monomer comprises a fatty acid moiety selected independently from the group consisting of conjugated diene fatty acids, conjugated di-yne fatty acids, conjugated
15 ene-yne fatty acids, and fatty acids containing sulphhydryl groups.

3. A stable perfluorochemical emulsion as is claimed in claim 1 wherein such a phospholipid monomer is a photo-
20 polymerizable phospholipid monomer.

4. A stable perfluorochemical emulsion as is claimed in claim 1 wherein such a phospholipid monomer is selected from the group consisting of phosphatidyl-L-cholines,
25 phosphatidyl-L-serines, phosphatidyl-L-ethanolamines, and mixtures thereof.

5. A stable perfluorochemical emulsion as is claimed in claim 1 in which such a phospholipid monomer is selected
30 from the group consisting of phosphatidyl-L-choline, phosphatidyl-L-serine, phosphatidyl-L-ethanolamine, and mixtures thereof, wherein both acyl chains of each such phospholipid monomer incorporate a conjugated di-yne fatty acid moiety.

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1 6. A stable perfluorochemical emulsion as is claimed
in claim 5 in which such a phospholipid monomer is
phosphatidyl-L-choline, wherein both acyl chains comprise
conjugated di-yne fatty acid moieties with the conjugated
5 di-yne bonds on the same carbons in both chains.

7. A stable perfluorochemical emulsion as is claimed
in claim 5 in which such a phospholipid monomer is phos-
phatidyl-L-serine, wherein both acyl chains comprise di-
10 yne fatty acid moieties with the conjugated di-yne bonds
on the same carbons in both chains.

8. A stable perfluorochemical emulsion as is claimed
in claim 5 in which such a phospholipid monomer is phos-
15 phatidyl-L-ethanolamine, wherein both acyl chains comprise
di-yne fatty acid moieties with the conjugated di-yne
bonds on the same carbons in both chains.

9. A stable perfluorochemical emulsion as is claimed
20 in claim 5 in which such a phospholipid monomer is phos-
phatidyl-L-choline, wherein both acyl chains comprise
conjugated di-ene fatty acid moieties with the conjugated
di-ene bonds on the same carbons in both chains.

25 10. A stable perfluorochemical emulsion as is claimed
in claim 5 in which such a phospholipid monomer is phos-
phatidyl-L-serine, wherein both acyl chains comprise di-
ene fatty acid moieties with the conjugated di-ene bonds
on the same carbons in both chains.

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11. A stable perfluorochemical emulsion as is claimed
in claim 5 in which such a phospholipid monomer is phos-
phatidyl-L-ethanolamine, wherein both acyl chains comprise
di-ene fatty acid moieties with the conjugated di-ene
35 bonds on the same carbons in both chains.

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1 12. A stable perfluorochemical emulsion as is claimed
in claim 5 in which such a phospholipid monomer is phos-
phatidyl-L-choline, wherein both acyl chains comprise ene-
yne fatty acid moieties with the conjugated ene-yne bonds
5 on the same carbons in both chains.

13. A stable perfluorochemical emulsion as is claimed
in claim 5 in which such a phospholipid monomer is phos-
phatidyl-L-serine, wherein both acyl chains comprise ene-
10 yne fatty acid moieties with the conjugated ene-yne bonds
on the same carbons in both chains.

14. A stable perfluorochemical emulsion as is claimed
in claim 5 in which the phospholipid is phosphatidyl-L-
15 ethanolamine, wherein both acyl chains comprise ene-yne fatty
acid moieties with the conjugated ene-yne bonds on the same
carbons in both chains.

15. A stable perfluorochemical emulsion as is claimed
20 in claim 1 in which the perfluorochemical particle comprises
a perfluorochemical selected from the group consisting of
perfluorodecalin, perfluorotertiary-amine, and isoquinolidine
perfluorochemical derivatives, and mixtures thereof.

25 16. A stable perfluorochemical emulsion as is claimed
in claim 1 in which the perfluorochemical particle comprises
a perfluorochemical selected from the group consisting of
(a) perfluorodecalin and perfluorotri-n-propylamine; (b)
perfluorotri-n-propylamine and perfluorotri-n-butylamine;
30 (c) perfluoro-N,N'-dimethylcyclohexylmethylamine and
perfluorodecalin; (d) perfluoro-3,3,1,-tri-methylbicyclo
[3,3,1] nonane and perfluoro-N,N'-dimethylcyclohexylme-
thylamine; and (e) cis and trans perfluorodecalin.

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1 17. A stable perfluorochemical emulsion as is claimed
in claim 1 in which the polymeric vesicles have a diameter
of up to about 0.3 micron.

5 18. A stable perfluorochemical emulsion as is claimed
in claim 17 in which the polymeric vesicles have a diameter
of about 0.15 to about 0.3 micron.

10 19. A stable perfluorochemical emulsion as is claimed
in claim 18 in which the vesicles have a diameter of about
0.2 micron.

20 20. A stable perfluorochemical emulsion as is claimed
in claim 1 in which the perfluorochemical particle comprises
15 perfluorodecalin and perfluorotri-n-propylamine, and the
phospholipid monomer is phosphatidyl-L-choline, wherein
both acyl chains comprise conjugated di-yne fatty acid
moieties with the conjugated di-yne bonds on the same
carbons in both chains, and the polymeric vesicle diameter
20 is from about 0.15 to about 0.3 micron.

25 21. A stable perfluorochemical emulsion in a physio-
logically acceptable aqueous medium comprising perfluoro-
chemical particles contained within stabilized vesicles,
25 the stabilized vesicles comprising a biocompatible polymer
formed by coating the perfluorochemical particles with a
phospholipid monomer selected from the group consisting of
phosphatidyl-L-cholines, phosphatidyl-L-serines, phospha-
tidyl-L-ethanolamines, and mixtures thereof, and polymerizing
30 the monomers.

35 22. A stable perfluorochemical emulsion as is claimed
in claim 21 wherein both acyl chains of the phospholipid
monomer comprise fatty acid moieties selected from the
35 group of conjugated di-yne fatty acids, conjugated di-ene

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1 fatty acids, and conjugated ene-yne fatty acids, wherein
the unsaturated bonds are on the same carbons in both acyl
chains.

5 23. A method for producing a stable perfluorochemical
emulsion, the method comprising the following steps:

(a) combining a perfluorochemical and a phos-
pholipid monomer in the absence of a polymerization initiator
to form a mixture;

10 (b) homogenizing the mixture in an aqueous
medium to form an emulsion comprising perfluorochemical
particles of a desired size coated with the phospholipid
monomer; and

(c) exposing the emulsion to a polymerization
15 initiator to cause such phospholipid monomer coatings to
polymerize to thereby form biocompatible phospholipid
polymeric vesicles, the emulsion comprising such stabilized
phospholipid polymer vesicles containing the perfluoro-
chemical particles.

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24. The method of claim 23 further comprising removing
oxygen from the perfluorochemical/phospholipid monomer
mixture before the mixture is homogenized in the aqueous
medium.

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25. The method of claim 23 wherein the phospholipid
monomer is a photopolymerizable phospholipid monomer.

26. The method of claim 23 wherein the phospholipid
30 monomer comprises a fatty acid moiety selected independently
from the group consisting of conjugated di-ene fatty acids,
conjugated di-yne fatty acids, conjugated ene-yne fatty
acids, fatty acids containing sulphydryl groups, and mixtures
thereof.

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1 27. The method of claim 23 wherein the phospholipid
monomer is selected from the group consisting of phosphatidyl-L-cholines, phosphatidyl-L-serines, phosphatidyl-L-ethanolamines, and mixtures thereof.

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 28. The method of claim 23 wherein the phospholipid
monomer is selected from the group consisting of phosphatidyl-L-choline, phosphatidyl-L-serine, phosphatidyl-L-ethanolamine, and mixtures thereof, and both acyl chains
10 in each such phospholipid comprise a conjugated di-yne fatty acid moiety.

 29. The method of claim 23 wherein the phospholipid
is phosphatidyl-L-choline, and both acyl chains of the
15 phosphatidyl-L-choline comprise conjugated di-yne fatty acid moieties with the conjugated di-yne bonds on the same carbons in both chains.

 30. The method of claim 23 wherein the phospholipid
20 is phosphatidyl-L-serine, and both acyl chains of the phosphatidyl-L-serine comprise di-yne fatty acid moieties with the conjugated di-yne bonds on the same carbons in both chains.

25 31. The method of claim 23 wherein the phospholipid
is phosphatidyl-L-ethanolamine, and both acyl chains of the phosphatidyl-L-ethanolamine comprise di-yne fatty acid moieties with the conjugated di-yne bonds on the same carbons in both chains.

30
 32. The method of claim 23 wherein the phospholipid
is selected from the group consisting of phosphatidyl-L-choline, phosphatidyl-L-serine, phosphatidyl-L-ethanolamine, and mixtures thereof, and both acyl chains in each such
35 phospholipid comprise a conjugated di-ene fatty acid moiety.

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1 33. The method of claim 23 wherein the phospholipid
2 is phosphatidyl-L-serine, and both acyl chains comprise
3 di-ene fatty acid moieties with the conjugated di-ene
4 bonds on the same carbons in both chains.

5

6 34. The method of claim 23 wherein the phospholipid
7 is phosphatidyl-L-ethanolamine, and both acyl chains comprise
8 di-ene fatty acid moieties with the conjugated di-ene
9 bonds on the same carbons in both chains.

10

11 35. The method of claim 23 wherein the phospholipid
12 is phosphatidyl-L-choline, and both acyl chains comprise
13 conjugated ene-yne fatty acid moieties with the conjugated
14 ene-yne bonds on the same carbons in both chains.

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16 36. The method of claim 23 wherein the phospholipid
17 is phosphatidyl-L-serine, and both acyl chains comprise
18 ene-yne fatty acid moieties with the conjugated ene-yne
19 bonds on the same carbons in both chains.

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21 37. The method of claim 23 wherein the phospholipid
22 is phosphatidyl-L-ethanolamine, and both acyl chains comprise
23 ene-yne fatty acid moieties with the conjugated ene-yne
24 bonds on the same carbons in both chains.

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26 38. The method of claim 23 wherein the perfluoro-
27 chemical is selected from the group consisting of perfluoro-
28 decalin, perfluorotertiary-amine, isoquinolidine perfluoro-
29 chemical derivatives, and mixtures thereof.

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31 39. The method of claim 38 wherein the perfluoro-
32 chemical is a mixture of perfluorodecalin and perfluorotri-
33 n-propylamine.

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INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/00985

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
INT. CL. ⁴ A61K 37/22 A61K 31/74		
US. CL. 424/78		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
U.S.	514/315, 672, 776 424/78	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹		
Category [*]	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	US, A, 4,252,827 (YOKOYAMA ET AL) 24 FEBRUARY 1981. SEE ENTIRE DOCUMENT.	1-22
X	US, A, 3,962,439 (YOKOYAMA ET AL) 08 JUNE 1976. SEE ENTIRE DOCUMENT.	1-22
A	US, A, 4,569,784 (MOORE) 11 FEBRUARY 1986 NOTE COL. 3, (LINE 25) TO COL. 5, (LINE 50) FOR SYNTHESIS.	23-39
A	US, A, 4,443,480 (CLARK, JR) 17 APRIL 1984 NOTE COL. 3, (LINE 67) TO COL. 7 (LINE 40) FOR COMPOSITION.	1-22
A	US, A, 4,285,928 (WADA ET AL) 25 AUGUST 1981 NOTE COL. 12 (LINE 1 ET SEQ.) FOR COMPOSITION	1-22
Y	US, A, 4,814,446 (ERNER) 21 MARCH 1989 NOTE COL. 3 (LINE 51) TO 32 FOR COMPOSITION.	1-22
X	US, A, 4,497,829 (SLOVITER) 05 FEBRUARY 1985	1-22
Y	NOTE COL. 3 (LINE 14) TO COL. 5, (LINE 40) FOR COMPOSITION AND SYNTHESIS.	25-39
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>[*] Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
11 APRIL 1989	12 JUN 1989	
International Searching Authority	Signature of Authorized Officer	
ISA/US	C. AZPURU	